APPENDIX C

relation to its target have been developed. The basis for optimism and anticipated change in clinical trials methodology extends from emerging understanding of the basis for cancer incidence and progression. Cancer arises from genetic lesions that cause an excess of cell growth or division, with inadequate cell death (Chap. 82). In addition, failure of cellular differentiation results in altered cellular position and capacity to proliferate while cut off from normal cell regulatory signals. An overall schema for understanding cancer progression can be seen in Fig. 84-2. Normally, cells in a differentiated state are stimulated to enter the cell cycle from a quiescent state, or "G0," or continue after completion of a prior cell division cycle in response to environmental cues, including growth factor and hormonal signals. Cells progress through G1 and enter S phase after passing through "checkpoints," which are biochemically regulated transition points, to assure that the genome is ready for replication. One important checkpoint is mediated by the p53 tumor-suppressor gene product, acting through its upregulation of the $p21^{WAF1}$ inhibitor of cyclin-dependent kinase (CDK) function, acting on CDKs 4 or 6. These molecules can also be inhibited by the $p16^{INK4A}$ and $p27^{KIP1}$ CDK inhibitors and, in turn, are activated by cyclins of the D family (which appear during G1) and the proper sequence of regulatory phosphorylations. Activated CDKs 4 or 6 phosphorylate and thus inactivate the product of the retinoblastoma susceptibility gene, pRb, which in its nonphosphorylated state complexes with transcription factors of the E2F family. Phosphorylated pRb releases E2Fs, which activate genes important in completing DNA replication during S phase, progression through which is promoted by CDK2 acting in concert with cylins A and E. During G2, another checkpoint occurs, in which the cell assures the completion of correct DNA synthesis. Cells then progress into M phase under the influence of CDK1 and cyclin B. Cells may then go on to a subsequent division cycle or enter into a quiescent, differentiated state.

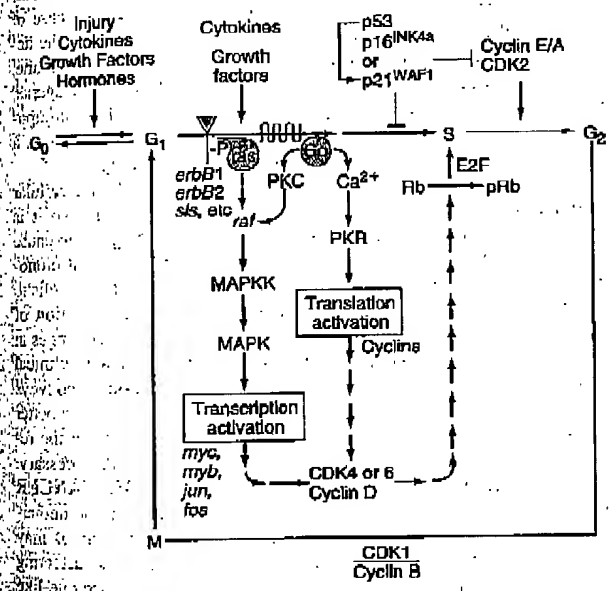

FIGURE 84-2 Basis for neoplastic growth and progression. Normally cells are stimulated to enter a proliferating state through the action of growth factors, positional signals, or cytokines. Cells enter G1 under the influence of normal signaling pathways including tyrosine kinase receptors coupled to ras protooncogenes or seven-transmembrane receptors coupled through heterotrimeric guanine nucleotide binding (G) proteins, especially G_q linked to calcium- and lipid-mediated signaling pathways through protein kinase C (PKC). Cells activate transcription and translation of key regulatory molecules such as the cyclins, which activate cyclin-dependent kinases (CDKs) 4 or 6. Phosphorylation of the retinoblastoma susceptibility protein (pRb) causes release of E2F transcription factors to an active state, promoting the transcription of multiple genes allowing progression through S phase, where DNA is replicated. Tumor cells possess activated oncogenes such as *erbB1*, *erbB2*, and *sis* or mutated *ras* gene products that are tonically activated, thus driving proliferation autonomously. *Raf* and the mitogen-activated kinases, MAPKK and MAPK, amplify the growth signal in a kinase cascade. "Brakes" to cell cycle progression include the p53-mediated G1 to S phase checkpoint, mediated by the CDK inhibitors p16INK4a and p21WAF1. Progression through S phase is also promoted by CDK2 acting in concert with cyclins E and A, and initiation of cell division is governed by the action of CDK1 acting in concert with cyclin B.

Also shown in Fig. 84-2 are the sites of action of protooncogenes, regulators of cellular proliferation that, in an active state, promote cell growth, and whose deregulation produces oncogenes, originally discovered as the genes encoded by tumor-forming viruses in animals. Oncogenes can be divided into two families: (1) those that act in the cytoplasm to disrupt normal growth factor–related signaling, including *ras*, *raf*, and the tyrosine kinases of the *src* and *erbB* or *sis* families; and (2) nuclear oncogenes, including *jun*, *fos*, *myc*, and *myb*, that act to alter transcriptional control of cassettes of genes. In contrast, tumor-suppressor genes, including p53 and pRb, act as cellular "brakes" whose normal function is to inhibit or prevent unregulated cellular growth. The capacity to divide indefinitely is provided by activation of *telomerase*, which allows continued replication of chromosomes by addressing the unique need of chromosome ends to be continually renewed to a proper length to allow normal mitosis. The capacity to invade and metastasize is conveyed by elaboration of *matrix metalloproteases* and *plasminogen activators* and the capacity to recruit host stromal cells at the site of invasion through tumor-induced *angiogenesis*.

As will become apparent below, currently used drugs for the treatment of cancer focus principally on the proximate biochemistry of nucleic acid and mitotic spindle structure or function. Drugs of the future may seek to replace lost function of tumor-suppressor genes; counter the action of activated oncogenes; influence the capacity of cells to die; prevent normal chromosomal end replication; actually infect cells with viruses designed to replicate in the milieu of the cancer but not the normal cell; cause differentiation of cells with exit from the cell cycle by activating the appropriate genes; and utilize immunologic strategies, including antibodies and engineered cells to be directed at novel proteins expressed on the surface of cancer cells.

BIOLOGIC BASIS FOR CANCER CHEMOTHERAPY

The classic view of how cancer chemotherapeutic agents cause regressions of tumors focused on models such as the L1210 murine leukemia system, where cancer cells grow exponentially after inoculation into the peritoneal cavity of an isogenic mouse. The interaction of drug with its biochemical target in the cancer cell was proposed to result in "unbalanced growth" that was not sustainable and therefore resulted in cell death, directly as a result of interacting with the drug's proximal target. Agents could be categorized (Fig. 84-3) as cell cycle–active, phase-specific (e.g., antimetabolites, purines, and pyrimidines in S phase; vinca alkaloids in M), and phase-nonspecific agents (e.g., alkylators, and antitumor antibiotics including the anthracyclines, actinomycin, and mitomycin), which can injure DNA at any phase of the cell cycle but appear to then block in G2 before cell division at a checkpoint in the cell cycle. Cells arrested at a checkpoint may repair DNA lesions. Checkpoints have been defined at the G1 to S transition, mediated by the tumor-suppressor gene p53 (giving rise to the characterization of p53 as a "guardian of the genome"); at the G2 to M transition, mediated by the *chk1* kinase influencing the function of CDK1; and during M phase, to ensure the integrity of the mitotic spindle. The importance of the concept of checkpoints extends from the hypothesis that repair of chemotherapy-mediated damage can occur while cells are stopped at a checkpoint; therefore, manipulation of checkpoint function emerges as an important basis of affecting resistance to chemotherapeutic agents.

Resistance to drugs was postulated to arise either from cells not being in the appropriate phase of the cell cycle or from decreased uptake, increased efflux, metabolism of the drug, or alteration of the target, e.g., by mutation or overexpression. Indeed, the *p170PGP*

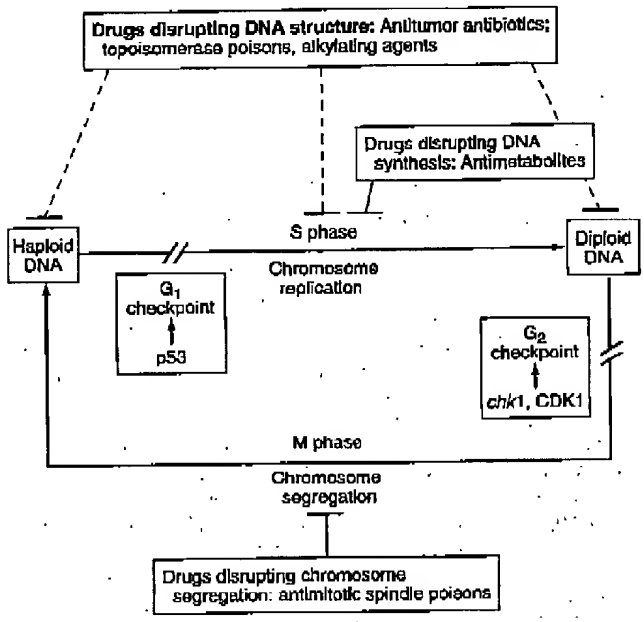

FIGURE 84-3 Location of drug action in the cell cycle. Cancer chemotherapy agents can be broadly described as phase-specific agents acting in S (antimetabolites) and M (spindle poisons) phase, respectively, and phase-nonspecific agents that injure their targets throughout the cycle but cause arrest of cell cycle progression at "checkpoints." The G1 checkpoint is mediated through p53 acting on CDKs 4,6, and 2, and the G2 checkpoint is mediated in part by the *chk1* kinase acting on CDK1.

(p170 P-glycoprotein; *mdr* gene product) was recognized from experiments with cells growing in tissue culture as mediating the efflux of chemotherapeutic agents in resistant cells. Certain neoplasms, particularly hematopoietic tumors, have an adverse prognosis if they express high levels of p170PGP, and modulation of this protein's function has been attempted by a variety of strategies.

Combinations of agents were proposed to afford the opportunity to affect many different targets or portions of the cell cycle at once, particularly if the toxic effects for the host of the different components of the combination were distinct. Combinations of agents were actually more effective in animal model systems than single agents, particularly if the tumor cell inoculum was high. This thinking led to the design of "combination chemotherapy" regimens, where drugs acting by different mechanisms (e.g., an alkylating agent plus an antimetabolite plus a mitotic spindle blocker) were combined. Particular combinations were chosen to emphasize drugs whose individual toxicities to the host were, if possible, distinct.

This view of cancer drug action is grossly oversimplified. Most tumors do not grow in an exponential pattern but rather follow Gompertzian kinetics, where the rate of tumor growth decreases as tumor mass increases (Fig. 84-1). Thus, a tumor has quiescent, differentiated compartments; proliferating compartments; and both well-vascularized and necrotic regions. Also, cell death is itself now understood to be a closely regulated process. *Necrosis* refers to cell death induced, for example, by physical damage with the hallmarks of cell swelling and membrane disruption. *Apoptosis*, or programmed cell death, refers to a highly ordered process whereby cells respond to defined stimuli by dying, and it recapitulates the necessary cell death observed during the ontogeny of the organism. *Anoikis* refers to death of epithelial cells after removal from the normal milieu of substrate, particularly from cell-to-cell contact. Cancer chemotherapeutic agents can cause both necrosis and apoptosis. Apoptosis is characterized by chromatin condensation (giving rise to "apoptotic bodies"); cell shrinkage; and, in living animals, phagocytosis by surrounding stromal cells without evidence of inflammation. This process is regulated either by signal transduction systems that promote a cell's demise after a certain level of insult is achieved or in response to specific cell-surface receptors that mediate cell death signals. Modulation of apoptosis by manipulation of signal transduction pathways has emerged as a basis for understanding the actions of currently used drugs and designing new strategies to improve their use.

The current view envisions that the interaction of a chemotherapeutic drug with its target causes or is itself a signal that initiates a "cascade" of signaling steps to trigger an "execution phase" where proteases, nucleases, and endogenous regulators of the cell death pathway are activated. Effective cancer chemotherapeutic agents are efficient activators of apoptosis through signal transduction pathways (Fig. 84-4). For example, in the cytokine-mediated pathway, exogenous ligands such as the Fas ligand (FasL) bind to cell-surface receptors (CD95; Fas), or tumor necrosis factor (TNF) or its homologue Apo2L binds to its cognate receptors and directly recruits accessory molecules to activate a protease cascade (utilizing members of the caspase family of cysteine *asp*artyl prote*ases*), resulting in apoptosis. In a second pathway, growth factor deprivation elicits poorly defined signals that result in protease activation. Chemotherapeutic agents create molecular lesions (in DNA or cellular membranes) as a consequence of combining with their respective molecular targets. These lesions are sensed by a cellular "damage sensor," whose molecular nature is unclear, which leads to mitochondrial damage. Release of mitochondrial factors (e.g., APAF1, cytochrome c) promotes the activation of another set of caspases. Damage to the plasma membrane, e.g., from free radicals generated by certain chemotherapeutic agents, leads to activation of acid sphingomyelinase to release lipid components including ceramides, which then promote apoptosis through a variety of pathways including direct mitochondrial damage.

While apoptotic mechanisms are important in regulating cellular proliferation and the behavior of tumor cells in vitro, in vivo it is unclear whether all of the actions of chemotherapeutic agents to cause cell death can be attributed to apoptotic mechanisms. Loss of clonogenic survival (conventionally detecting the capacity of a few cells to survive) may predict clinical value more reliably than detection of apoptotic changes in the majority of tumor cells. However, changes in molecules that regulate apoptosis are clearly correlated with clinical outcomes (e.g., *bcl2* overexpression in certain lymphomas conveys poor prognosis; proapoptotic *bax* expression is associated with a better outcome in ovarian carcinoma). Further efforts to understand the relationship of cell death and cell survival mechanisms will be necessary.

CHEMOTHERAPEUTIC AGENTS USED FOR CANCER TREATMENT Table 84-2 lists commonly used cancer chemotherapy agents and pertinent clinical aspects of their use. The drugs may be usefully grouped into three general categories: those affecting DNA, those affecting microtubules, and those acting at hormone-like receptors.

Direct DNA-Interactive Agents • ***Formation of covalent DNA adducts*** Alkylating agents as a class break down, either spontaneously or after normal organ or tumor cell metabolism, to reactive intermediates that covalently modify bases in DNA. This leads to cross-linkage of DNA strands or the appearance of breaks in DNA as a result of repair efforts. "Broken" or cross-linked DNA is intrinsically unable to complete normal replication or cell division; in addition, it is a potent activator of cell cycle checkpoints and signaling pathways that can activate apoptosis. As a class, alkylating agents share similar toxicities, including myelosuppression, alopecia, gonadal dysfunction, mucositis, and pulmonary fibrosis. They differ greatly in a spectrum of normal organ toxicities.

Nitrogen mustard (mechlorethamine) is the prototypic agent of this class, decomposing rapidly in aqueous solution to yield potentially a bifunctional carbonium ion. It must be administered shortly after preparation into a rapidly flowing intravenous line. It is powerful vesicant, and infiltration may be symptomatically ameliorated by infiltration of the affected site with 1/6 *M* thiosulfate. Even without infiltration, asep-

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